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Genetic and Morphometric Characterization of Mussels (Bivalvia: Mytilidae) From Mid-Atlantic Hydrothermal Vents

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Abstract. Mussels were collected from deep-sea hydrothermal vents along the Mid-Atlantic Ridge. Specimens from the Snake Pit site were previously identified genetically and anatomically as *Bathymodiolus puteoserpentis*, but the relationships of mussels from other sites (Logatchev and Lucky Strike) were unclear. Molecular genetic and morphological techniques were used to assess differences among these mussel populations. The results indicate that the range for *B. puteoserpentis* extends from Snake Pit to Logatchev, and that an unnamed second species, *B.* n. sp., occurs at Lucky Strike. Analysis of mitochondrial NADH dehydrogenase subunit 4 (ND4) revealed 13% sequence divergence between the two species. Nei's genetic distance (*D*) based on 14 allozyme loci was 0.112. A multivariate morphometric analysis yielded a canonical discriminant function that correctly identified individuals from these sites to species 95% of the time.

Introduction

Modioliform mussels that depend wholly or in part on symbiotic bacteria for their nutriment are common constituents of biological communities associated with deep-sea hydrothermal vents and cold-water sulfide/hydrocarbon seeps throughout the world. Eight species of these mussels were known to occur in vent and seep environments in the Atlantic and Pacific Oceans (Desbruyères and Segonzac, 1997). Recently, five new species, including one new genus, were described from hydrocarbon seeps in the Gulf of Mexico (GOM) (Gustafson *et al.*, 1998). The new GOM species were first identified in an allozyme study aimed at describing genetic diversity in these mytilids (Craddock *et al.*, 1995a). This study also recognized two genetically distinct populations of mussels from the Mid-Atlantic Ridge (MAR; Fig. 1). The degree of divergence between mussels from the Snake Pit (23°22′N) and Lucky Strike (37°17′N) localities (Nei's $D = 1.179$) led the authors to suggest that two distinct species occupied the MAR. Von Cosel and coworkers (1994) described the mussels from Snake Pit as a new species, *Bathymodiolus puteoserpentis*. On the basis of morphological comparisons, they subsequently suggested that mussels from Lucky Strike should be recognized as a distinct species, but to date the Lucky Strike mussels remain unnamed (von Cosel *et al.*, 1997). We refer to the second MAR species as *B.* n. sp. for the present purposes. Von Cosel *et al.* (1997, p. 146) also suggested that mussels from the southernmost MAR locality (Logatchev; 14°45′N) "belong to another new species, which is however extremely close to *B. puteoserpentis* . . . only an electrophoretic analysis could determine the distance between this new (*e.g.*, Logatchev) species and *B. puteoserpentis*."

The purpose of this study was to clarify evolutionary relationships among these MAR mussels. We examined mitochondrial DNA sequences and allozyme variation in new samples collected from Lucky Strike, Snake Pit, and Logatchev during July 1997 (Fig. 1; Table 1). We clearly show that the Logatchev population and *B. puteoserpentis* are genetically identical and do not warrant separation as distinct species. In addition, we clarify the genetic distances between the two known Mid-Atlantic Ridge species.

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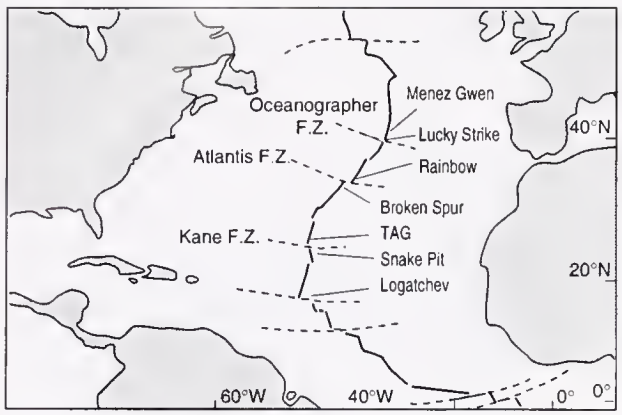

Figure 1. Geographic locations of *Bathymodiolus* collection sites along the Mid-Atlantic Ridge. Major fracture zones (F.Z.) are shown.

Materials and Methods

Specimens

Using the deep submergence vehicle *Alvin's* mechanical claw, samples were collected along the MAR (Fig. 1, Table I) in July 1997. Once aboard the support vessel *Atlantis*, we placed the specimens in 4°C filtered seawater. Mytilids were then either dissected or frozen whole. For dissected specimens, gill, mantle, adductor muscle, and tissue mass were frozen at −70°C in labeled bags; shells were then measured and labeled. All samples were brought back to Rutgers University on dry ice and stored at −80°C until they were prepared for genetic analysis.

DNA extraction, polymerase chain reaction, and sequencing

Whole cellular DNA was isolated by digesting 0.05–0.1 g of frozen tissue following a hexadecyl-trimethyl-ammonium bromide (CTAB) protocol (Doyle and Dickson, 1987), followed by a phenol extraction and ethanol precipitation (Sambrook *et al.*, 1989). Purified nucleic acids were rehydrated in 1X TE buffer (10 m*M* Tris-HCl, pH 7.5; 1 m*M* EDTA) to a final concentration of 100–500 ng/μl and stored for short periods at 4°C and for longer periods at −20°C.

An approximately 710-bp region of the mitochondria was amplified using primers designed to amplify ND4-L in fish. Arg BL (5′-CAA gAC CCT TgA TTT Cgg CTC A-3′) is in the tRNA arginine (Bielawski and Gold, 1996) and NAP 2H (5′-Tgg AgC TTC TAC gTg A/ggC TTT-3′) is within ND4 itself (Arevalo *et al.*, 1994). Comparison of the resulting sequences *via* BLASTN (Altschul *et al.*, 1990) and BLASTP (Altschul *et al.*, 1997) searches of GenBank sequences indicated that these primers amplified tRNA-Met, tRNA-Val, and the first third of ND4 in *Bathymodiolus*. The 50-μl amplification reaction contained 100–500 ng template DNA, 2.5 m*M* $MgCl_2$, 20 μ*M* dNTP (5 μ*M* each nucleotide), 0.4 μ*M* of each primer, 1.5 units *Taq* polymerase, and 5 μl 10× buffer (Promega, Madison, WI). The polymerase chain reaction (PCR) profile (95°C/45s, 56°C/45s, 72°C/min) continued for 35 cycles, with an initial denaturation at 95°C/2 min and a final extension at 72°C/7 min. Negative controls were included with each set of amplifications.

PCR product (5 μl) from each set of reactions was viewed on a 1% agarose gel stained with ethidium bromide, and the remaining 45 μl was extracted once with chloroform/isoamyl alcohol (24:1) and precipitated with 22.5 μl 8 *M* ammonium acetate (pH 5.8) and 90 μl cold 100% ethanol. Sequencing reactions were performed for each individual with both the heavy and light strand primers used for amplification. We used 60–70 ng of template DNA in a 10-μl sequencing reaction containing 4.25 μl FS mix (Applied Biosystems, Foster City, CA), 0.2 μ*M* primer, and sterile distilled H_2O. The cycle-sequencing profile (95°C/30 s, 50°C/15 s, 60°C/4 min) continued for 25 cycles. Electrophoretic separations of the sequencing reactions were performed on a Perkin-Elmer ABI 373 DNA sequencer (Foster City, CA).

The 507-bp ND4 sequence was edited and aligned in Auto Assembler (ver. 1.4.0, Applied Biosystems) and Sequence Navigator (ver. 1.0.1, Applied Biosystems). Trans-

Table 1

Mussel samples collected during July 1997 along the Mid-Atlantic Ridge (MAR)

Location	Latitude Longitude	*Alvin* Dive number	Depth (m)	Species	*n* Sequenced	*n* RFLP	*n* Allozymes	*n* Morphology
Lucky Strike	37°17′N 32°15′W	A3120	1710	*Bathymodiolus* n. sp.	5	11	27	123
Snake Pit	23°22′N 44°56′W	A3129	3480	*B. puteoserpentis*	3	9	30	40
Logatchev	14°45′N 44°58′W	A3133 A3131	3080	*B. puteoserpentis*	5	11	34	113

n is the number of individuals used for each type of analysis.

Table II

Enzyme buffer combinations used in allozyme electrophoresis

Enzyme	Loci	Buffer system
Aspartate aminotransferase	*Aat*	B
Isocitarte dehydrogenase	*Idh*	B
Leucine aminopeptidase	*Lap*	C and D
Malate dehydrogenase	*Mdh*-1, *Mdh*-2	A
Malic enzyme	*Me*	B
Mannose-6-phosphate isomerase	*Mpi*	D
D-Octopine dehydrogenase	*Opdh*	C
Peptidase LG, LGG	*Pep*-gl, *Pep*-lgg	A and D
Phosphoglucose isomerase	*Pgi*	A and D
Phosphoglucomutase	*Pgm*	B and D
6-Phosphogluconate dehydrogenase	*Pgd*	B and D
Superoxide dismutase	*Sod*	C

Buffer A (AP 6.0), B (TC 6.0), C (TVB 8.0) and D (TC 7.0).

lation and amino acid alignments were performed in ESEE (Cabot and Beckenbach, 1989). Genetic distances were calculated in MEGA (Kumar *et al.*, 1994). Due to a large bias in nucleotide base composition, pairwise nucleotide sequence divergence was estimated with the Tajima-Nei distance algorithm (Tajima and Nei, 1984). GenBank accession numbers are AF128534 and AF128533 for haplotypes A1 (*B.* n. sp.) and B1 (*B. puteoserpentis*), respectively.

Restriction fragment length polymorphism analysis

The sequences were used to identify a restriction enzyme, *Cac*8 I, which produced diagnostic restriction band patterns for the two mitochondrial haplotypes A1 and B1. ND4 product was obtained by PCR as described above; 10 μl was digested with 1 μl of *Cac*8 I and 2 μl of the appropriate buffer (New England Biolabs, Beverly, MA) and 7 μl sterile distilled water for a total volume of 20 μl for 1 h at 37°C. Digestions were separated by electrophoresis on a 2% agarose gel containing ethidium bromide. The gel was viewed and photographed under UV light.

Allozymes

About 0.2 g of tissue from the mantle was removed and homogenized in two volumes of grinding buffer (0.05 *M* Tris/20% glycerol, pH 8.0) and spun at 15,000 × *g* for 10 min to remove tissue debris. The supernatant fluid was inserted into a preformed well in a 12.5% starch gel (StarchArt, Smithville, TX). We used four buffer systems that were previously shown to resolve polymorphic enzymes in these mytilids (Craddock *et al.*, 1995a), Buffer A (AP 6), Buffer B (TC 6), Buffer C (TVB 8), and Buffer D (TC 7). Horizontal starch gels were used to assess variation at 14 loci (Table II) following standard protocols (Murphy *et al.*, 1990). To confirm allelic identities, samples were run on multiple gels after the loading order was varied.

Population genetic analyses were performed with programs BIOSYS-1 (Swofford and Selander, 1981) and DISPAN (Ota, 1993). Nei's *D* (Nei, 1978) was estimated from electromorph-frequency data. Agreement with random mating expectations was determined by the "exact HW test" (Guo and Thompson, 1992). *F* statistics were estimated by the method of Nei (Nei, 1977), and heterogeneity among samples was assessed by contingency chi-squared analysis.

Morphology

On board the support vessel, all specimens were measured using a hand-held electric caliper accurate to 0.01 mm. Six shell measurements (Fig. 2) were recorded: A, anterior length (the distance from the anterior shell margin to the anterior edge of umbo); G, ligament length; H, shell height; L, shell length; W, width of shell valves; and U, umbo length (the distance from the anterior edge of umbo to the posterior shell margin). Linear regressions and principal components analysis of morphological data were performed in JMP (SAS Institute, 1986), and discriminant analysis was performed in SAS (SAS Institute, 1997). All variables were natural log transformed prior to multivariate analyses.

Results

Mitochondrial DNA sequences

The DNA fragment used for this study included sequences for two transfer RNAs (200 bp) and the first third of the ND4 gene. The tandemly arranged tRNAs presented problems for alignment of the sequences. Thus for the present study, we used sequences only for the first 507 bp of the protein coding ND4 gene. Altogether, we examined sequences from 13 specimens collected at the three MAR locations (Table 1). Six unique ND4 sequences could be assigned to two major haplotypes (A and B) that corresponded to morphospecies *B.* n. sp. and *B. puteoserpentis*, respectively (Fig. 3). On average, the two major haplotypes differed at 13% of the 507 bp (Table III), including 14 inferred amino acid substitutions. Haplotype A included

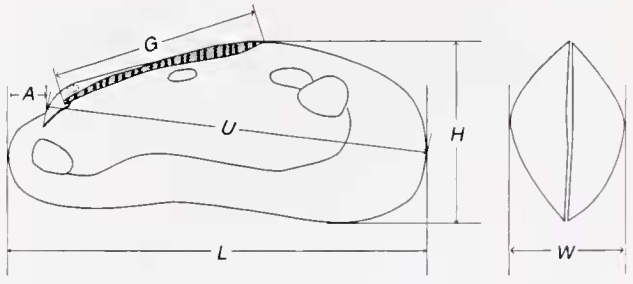

Figure 2. Diagram depicting shell measurements. A, anterior length (distance from anterior shell margin to anterior edge of umbo); H, shell height; L, shell length; G, ligament length; U, umbo length (distance from anterior edge of umbo to posterior shell margin); W, width of shell valves.

A.

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ATG TGT ATA GTG CTT TTA GGG GTT TTA GGG GTT CTA GAG GTA TCG GTT GCT GGC   54
TTT TCT TTG CTT GGG GTG CTA GCT ATG CCT TTG TTT CTG TCC GGA AGG TGC TCT  108
GAA ATA AAC GGT ATG TTT AAT TTG GAT TTT TCG GGG CAG ACT CTG GTA ATT CTT  162
AGA ATC TAT ATC AGT TTT TTA ATA TTA ATG GGG AGG GTC ACA GTG TCT CGG TTT  216
AGT GCA TTT AAT AGG CTT ATT GTT TGC ATT GGT GCT TTG TTG GTC GGT GCC TTT  270
AGT GTT AGG GTT ACT TTC CTT TTC TTC GTT CTT TTT GAG GGC GTT TTG TTT CCC  324
ACT TTG CTG TTA ATT GTT GGA TGG GGG TAC CAG CCG GAG CGT TTA CAG GCA GTT  378
GTT TAT ATA GTT ATT TAC ACT GTT ATA GGG TCC CTA CCC CTT TTA TAT GGC TTG  432
GGA AAA CTT TAT TTT CAT GAC GGG AGA GAC AAT TTA TTT AGG TTG GAA TTT GTT  486
CTT GAC AAA AGT ATT TTA AGG                                             507
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B.

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                          1111111111111112222222222223333344444444444455
          2345577899999900012344557888912335667778814456001122335789 00
          4254538513456925678518174379068581570691822510581769256442 12

A1 (3)    CGATCTCCTACTTCATATGCAGTGTGCGGCCGATTTCCACTTGTTTGAATCTAGAATTCT
A2 (1)    ............................................................C
A3 (1)    ...C........................................................

B1 (3,3) TTGCTCTTCGTCCAGCTCATGACACATAATTAGCGCTTGTCCTAGGAGCCTCGAGGGCT.
B2 (0,1) ..................................................C.........
B3 (0,1) ................................T.............................
AA Δ       *  *      *    * *   **    *  *** *   *                 *
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C.

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              11334556788896
              19263034904942

A1,2,3 (5)    GLLTSAVVVIVSAF

B1,2    (3,4) VFSSNTIMIVAAVV
B3      (0,1) ..........V...
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Figure 3. Mitochondrial ND4 haplotypes of the *Bathymodiolus* mussels on the Mid-Atlantic Ridge. Haplotype A corresponds to *B.* n. sp. and haplotype B to *B. puteoserpentis*. For haplotype A, numbers in parenthesis indicate the number of sequences for the Lucky Strike site. For haplotype B, the first number in parenthesis is the number from the Snake Pit site and the second is the number from the Logatchev site. (A) complete sequence for *B. puteoserpentis*, haplotype B1; (B) Nucleotide positions that differ between haplotypes; * indicates non-synonomous substitutions; (C) presumptive amino acid residues that differ between them.

three minor variants that differed by one (A1, A2) or two (A2, A3) synonymous substitutions. Haplotype B included variants that differed from each other by one synonymous substitution (B1, B2) and differed from a third (B3) by one non-synonymous substitution. The percentage sequence divergence within the two major haplotypes was 0.27%.

Population survey (PCR-RFLP)

Given small sample sizes, the mtDNA sequence information did not exclude the possibility of the alternative haplotype also occurring at these putatively monotypic localities. To increase the sample size, we used a six-base cutter enzyme, *Cac*8 I, that produced diagnostic fragment profiles for each major haplotype (Fig. 4). With the added RFLP information, our samples were increased to 12–16 individuals per locality (Table I), 31 specimens total. No additional variation was found within localities.

Allozymes

To quantify within-locality variation, we examined 14 allozyme loci (Table II) that previously were used to discriminate among the MAR mytilid species (Craddock *et al.*, 1995a). Hardy-Weinberg testing of 10 to 11 polymophic loci in each population revealed no significant deviations

Table III

Genetic distances and standard errors (in parentheses) between Mid-Atlantic Ridge Bathymodiolus *species*

	Lucky Strike	Snake Pit	Logatchev
Lucky Strike	0.0027	0.1325	0.1307
	(0.0023)	(0.0186)	(0.0184)
Snake Pit	**0.110**	0.0000	0.0020
	(0.085)	(0.0000)	(0.0020)
Logatchev	**0.114**	**0.000**	0.0027
	(0.088)	**(0.001)**	(0.0023)

Plain type: Average nucleic acid distances within and among populations for a 507-bp fragment of mitochondrial ND4. Boldface type: Nei's *D* based on 14 polymorphic allozyme loci.

from random mating expectations after application of a sequential Bonferroni adjustment (Rice, 1989). The multilocus observed heterozygosity (H_{obs}; Table IV) approximately equaled the expected value (H_{exp}), revealing no pattern of systematic deviation from random mating.

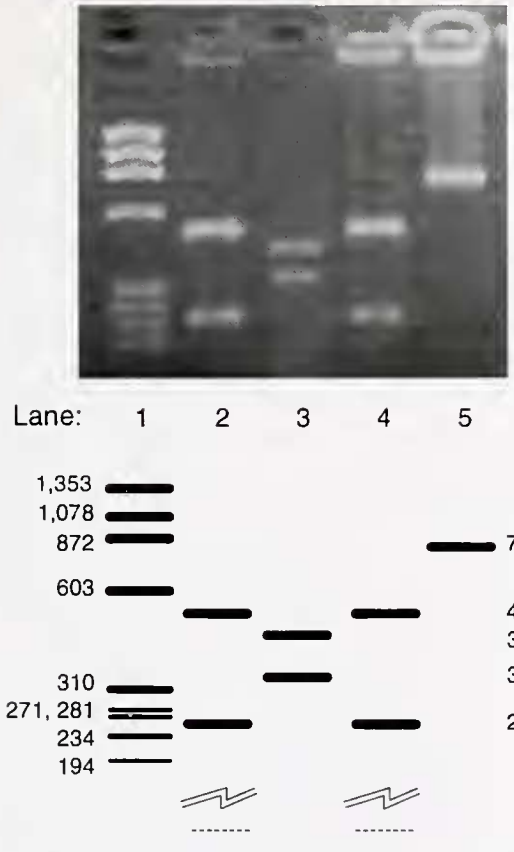

Figure 4. Restriction fragment banding pattern of the two Mid-Atlantic Ridge *Bathymodiolus* haplotypes upon digestion of the mitochondrial ND4 PCR fragment with *Cac* 8I. Lanes from left to right are (1) *Phi*-X *Hind* III ladder; (2) Snake Pit; (3) Lucky Strike; (4) Logatchev; (5) uncut PCR fragment.

Table IV

Allozyme diversity in Mid-Atlantic Ridge Bathymodiolus *species*

Site	n	P_{95}	H_{obs}	H_{exp}
Lucky Strike	26.1	64.29	0.256 (SE0.069)	0.242 (SE0.062)
Snake Pit	29.7	50	0.150 (SE0.045)	0.161 (SE0.052)
Logatchev	33.2	42.86	0.133 (SE0.043)	0.146 (SE0.049)

n = mean sample size; P_{95} = percentage of polymorphic loci (95% criteria); H_{obs} and H_{exp} = observed and expected mean heterozygosity per sample (standard errors in parentheses).

Craddock *et al.* (1995a) did not identify intraspecific polymorphisms in an earlier study that examined 26 gene loci in two mussels from Lucky Strike and two from Snake Pit. Techniques used in this earlier study were optimized to show interspecific differences between more divergent mussel species from the Gulf of Mexico, and thus failed to identify intraspecific variation within the MAR samples. The present study used techniques that were optimized to show the interspecific allozyme differences between *B. puteoserpentis* and *B.* n. sp. Using a subset of 14 loci reported by Craddock *et al.* (1995a), we found a high percentage of polymorphism in each population sample (Table IV). Interspecific monomorphic loci were not examined in this study; thus, the present estimates of polymorphism and heterozygosity should not be used in comparative analyses of genetic diversity.

Nei's (1978) genetic distances (*D* values) were estimated between the mussel populations from the three locations (Table III). The *D* value between the Logatchev and Snake Pit samples of *B. puteoserpentis* was 0.00, and the average *D* value between the two *B. puteoserpentis* samples and *B.* n. sp. was 0.112. The interspecific value is much lower than that reported by Craddock *et al.* (1995a) because the smaller sample sizes used in the previous study resulted in the mistaken interpretation of intraspecific differences as interspecific. We are currently developing a larger array of allozyme and DNA genetic markers to undertake a hierarchical analysis of genetic diversity and intraspecific gene flow among MAR mussel populations.

Morphology

We performed a principal components analysis to assess overall character correlations (Table V) for the six shell characters measured (Fig. 2). The first two rotated axes (PC-1, PC-2) encapsulated 98.5% of the total variance. Covariates of overall size loaded highly on PC-1, and the length of shell anterior to the beak (A) loaded highly on PC-2. A PC-2 component based on similar character loadings was useful for distinguishing among modioliform species from the Gulf of Mexico (Gustafson *et al.*, 1998). Nevertheless, considerable overlap remains between the

Table V

Correlation matrix of six morphological characters (natural log transformed) from Mid-Atlantic Ridge Bathymodiolus *species*

Variable	L	H	W	A	G	U	PC-1	PC-2
L	1.000	0.971	0.973	0.897	0.986	0.997	0.825	−0.527
H		1.000	0.964	0.921	0.959	0.962	0.710	−0.594
W			1.000	0.910	0.966	0.966	0.752	−0.571
A				1.000	0.891	0.872	0.507	−0.847
G					1.000	0.984	0.830	−0.530
U						1.000	0.853	−0.482

The PC-1 and PC-2 columns represent loadings of each character from the first two Varimax rotated principal component axes. L = shell length, H = shell height, W = width of shell valves, A = anterior length, G = ligament length, and U = umbo length (see Fig. 4).

two MAR species (Fig. 5). To develop a more useful morphological diagnostic, we performed a stepwise discriminant analysis with the six shell measurements. Four variables, when used in a canonical discriminant analysis, result in correct identification of individuals with respect to species 95% of the time (Fig. 6). The discriminant equation for the canonical value, C, is

$$C = 1.12 \ln A - 38.16 \ln U + 34.39 \ln L + 4.71 \ln H.$$

Values of $C < 5.5$ were classified as *B.* n. sp., and $C > 6.5$ are *B. puteoserpentis.* Values in the range $5.5 \geq C > 6.5$ could not be assigned to either species, due to the 5% overlap in canonical value distributions. In addition, two *B.* n. sp. individuals had values greater than 6.5.

Discussion

Von Cosel *et al.* (1997) raised doubts about the specific status of newly discovered mussels from the Logatchev locality on the Mid-Atlantic Ridge; the misgivings were based on the morphological affinity between these mussels and *B. puteoserpentis* from the Snake Pit locality. The authors' recommendation of a genetic analysis to resolve relationships between these populations was warranted, as the present study identified no substantial genetic divergence between the two. Despite the observation of considerable allozyme polymorphism within the two populations, Nei's *D* between them was zero. The mtDNA haplotypes found at the two sites also revealed no evidence for differentiation; sequence divergence averaged 0.2%. Finally, a multivariate analysis of shell morphometrics provided no

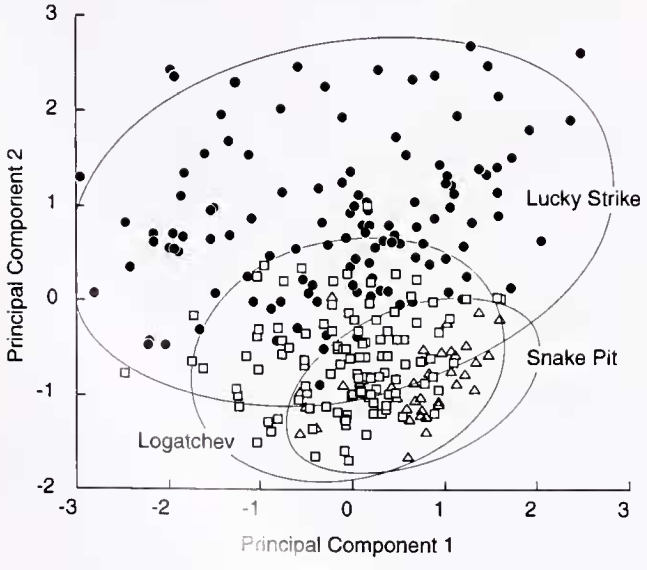

Figure 5. Scatter plot of the first two Varimax rotated principal components (PC-1 and PC-2) for shell measurements of Mid-Atlantic Ridge *Bathymodiolus.* Ellipses encompass 95% confidence limits for each species. Open triangles, *Bathymodiolus puteoserpentis* from the Snake Pit locality; open squares, *Bathymodiolus puteoserpentis* from the Logatchev locality; closed circles, *Bathymodiolus* n.sp. from the Lucky Strike.

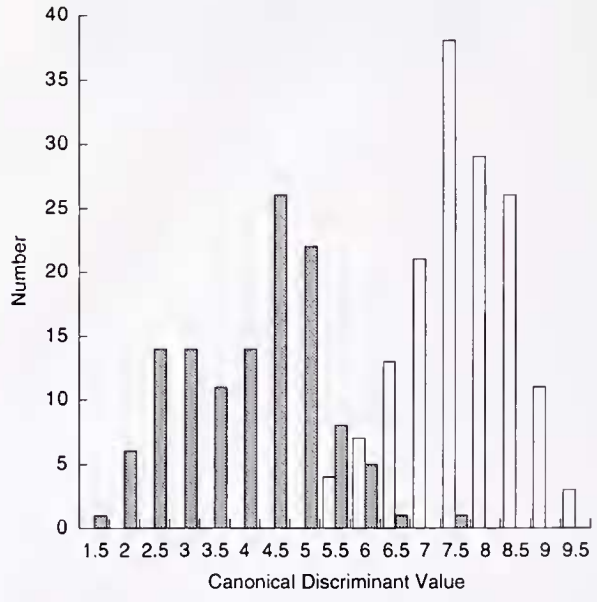

Figure 6. Histogram of the canonical discriminant value for Mid-Atlantic Ridge *Bathymodiolus. Bathymodiolus puteoserpentis* = white bars; *Bathymodiolus* n.sp. = shaded bars.

basis for separating the two *B. puteoserpentis* samples. Clearly, mussels from the Logatchev locality should be considered *B. puteoserpentis*.

The present genetic and morphometric analyses are consistent with recognition of *B. puteoserpentis* and *B.* n. sp. as distinct species. These mytilids typically express extreme morphological variability that may result from living in microhabitats that differ greatly in temperature and water chemistry (von Cosel *et al.*, 1997). Although we found some overlap in morphometric characteristics of the two species, discriminant analysis provided a useful basis for separating them 95% of the time. Dubious individuals, and possibly intergrades, can be identified with the application of molecular methods. First, mtDNA haplotypes based on a 507-bp portion of the mitochondrial ND4 gene unequivocally distinguished between the two species. Sequence divergence between *B. puteoserpentis* and *B.* n. sp. was 13%. This value is consistent with interspecific levels of divergence found in parallel studies of other deep-sea modioliform species (Maas *et al.*, unpubl. data).

In contrast, the present degree of allozyme divergence between *B. puteoserpentis* and *B.* n. sp. ($D = 0.112$) was low compared to an earlier report ($D = 1.179$) for mussels from Snake Pit (*B. puteoserpentis*) and Lucky Strike (*B.* n. sp.), (Craddock *et al.*, 1995a). This discrepancy can be explained by the larger sample sizes in the present study. Although the "number of individuals to be used for estimating genetic distances can also be very small if the genetic distance is large and the average heterozygosity of the two species compared is low" (Nei, 1978, p. 583), these criteria were not met in the light of present information. We found that heterozygosity was high and genetic distance was low; therefore, the earlier study confounded intraspecific variation with interspecific differences.

We are currently using the ND4 haplotypes to better define the ranges of *B. puteoserpentis* and *B.* n. sp. from additional MAR localities (Fig. 1). Preliminary data indicate that *B.* n. sp. is present at northern localities including the Menez Gwen, Lucky Strike, and Rainbow hydrothermal vent fields, and that *B. puteoserpentis* occurs at southern localities including Snake Pit and Logatchev. Preliminary mitochondrial information indicates that both species may occur at an intermediate locality, Broken Spur; however, additional work with allozymes is needed to determine whether the two species intergrade at this site. Several ecological factors contribute to separation of the two MAR species. The northern localities with *B.* n. sp. are at shallower depths, ranging from 869 m to 2303 m; the southern localities, with *B. puteoserpentis*, are at greater depths, ranging from 3080 m to 3480 m (Table 1). Ridge offsets associated with transform faults create barriers to dispersal and prevent mixing of some deep-sea vent species (Van Dover, 1990). Three transform faults (Oceanographer at 35°N, Atlantis at 30°N, and Kane at 24°N) may slow dispersal between the northern and southern localities. It is worth noting that the Broken Spur site (29°10′N) occurs between the Atlantis and Kane Transform Faults, and that it may contain both MAR species, but mussels were very rare at Broken Spur in 1997. We found no mussels at the nearby TAG hydrothermal mound, although a few were previously observed there. It is possible that this intermediate zone (35°N to 24°N) provides an unsuitable habitat for either species of mussel, although other vent-endemic fauna (*e.g.*, *Rimicaris exoculata*) are abundant at these sites (Van Dover, 1995).

Ridge offsets do not appear to provide significant barriers to dispersal for most vent organisms with a free-swimming larval stage (Vrijenhoek, 1997). For example, in the eastern Pacific, the mussel *Bathymodiolus thermophilus* and the vesicomyid clam *Calyptogena magnifica* showed relatively little differentiation and high rates of gene flow among sites along the East Pacific Rise and Galapagos Rift (Craddock *et al.*, 1995b; Karl *et al.*, 1996). All the eastern Pacific sites occurred at about the same depth (~2500 m), however. Because we have no reason to believe that the Mid-Atlantic mussels' dispersal abilities differ from those of *B. thermophilus*, it is possible that depth may provide a more fundamental barrier to dispersal than ridge offsets. We are currently engaged in a more detailed study of gene flow and dispersal in the MAR mytilids.

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